THE TEST OF A PURE SPECIES OF ŒNOTHERA.[1]

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There is probably no group of plants the genetic behavior of which has received so much study as the species of *Œnothera*. No group of plants is more prominently before the attention of experimental plant morphologists, and yet to many botanists it may appear that no group has yielded less of satisfaction. Among the workers with these forms there is the widest divergence of opinion, and of general conclusions there is little to show for the time that has passed since the appearance of "Die Mutationstheorie" in 1901 and the many years of study that De Vries devoted to the group previous to this date.

Can we find the point around which the difficulties cluster most thickly or from which the varied interpretations diverge most sharply? And, finding such a point can we formulate lines of experimentation that may clear the confusion of assumptions from which the various workers have proceeded to follow the lines of study that seemed to them to lead towards the light? To the writer the center of the difficulties lies in the fact that we have no accepted tests for the genetic purity of an *Œnothera* species.

By the genetic purity of a species we mean such a constitution of the germ plasm that a form is able to produce gametes of one type only for each sex. That is to say all male gametes of the form should have the same germinal constitution and thus be physiologically and morphologically equivalent, and all female gametes likewise should be of the same type. The male and female gametes may, however, differ in their respective effects upon the characters of a succeeding generation as shown by the marked differences exhibited by certain reciprocal crosses, for example, the reciprocals between *biennis* and *muricata,* or between *biennis* and *franciscana*

[1] Genetical Studies on Œnothera—VI.

(De Vries '13, Davis '14). The zygotes of a pure species must be uniform since the gametes of each sex are respectively similar, and a pure species, to employ that convenient expression of Bateson's, is therefore homozygous.

It has generally been held that no further proof of the genetic purity of a species is necessary than the established fact that it will "breed true," and I venture to believe that at present most workers among the œnotheras regard this test as entirely sufficient to establish the character of any material with which they work. If any line of *Œnothera* breeds true in large cultures it is confidently regarded as homozygous. Should a line fail to breed true to any considerable degree it is stamped as a hybrid if the investigator inclines towards the methods of analysis characteristic of the Mendelian school. Those who believe in mutations are so fully content with this test that to them a form need breed only reasonably true to pass as a pure species and the departures from the type, called mutations, are interpreted as due to modifications of the germ plasm not, however, the result of hybridism.

If a line of *Œnothera* fails to breed true to a very considerable degree and thus becomes suspected of a hybrid constitution, few workers would think of using it as favorable material for experimental studies to test the mutation theory. It is the lines which breed reasonably true that chiefly form the subjects of *Œnothera* discussions with reference to the theory of mutation. Such a line is the *Lamarckiana* of De Vries's cultures which when grown in large numbers in selfed families appears uniform except for certain small proportions of individuals, "mutants," which stand out clearly from the mass with distinctive characters that are readily recognized and may be clearly described. It is important to note that these new types are not connected by intergrading forms with the parent *Lamarckiana* and that they appear in successive generations of *Lamarckiana* with certain degrees of regularity.

More impressive than this history of *Lamarckiana* which has flowers open-pollinated, and consequently likely in Nature to have been crossed by insects, is the behavior reported for certain lines of *Œnothera* with flowers close-pollinated in the bud, a condition that obviously gives their own pollen the first chance to function and thus greatly reduces the probabilities of cross-pollination. Such a plant is the *biennis* of Holland and other parts of Europe, a type of especial interest not only for its clear morphological characters but also because there is good reason for believing the line to be very old. This plant forms a large population in Holland with no near relatives and must have lived there for many years to have so thoroughly established itself. Indeed it seems probable that this *Œnothera,* the Dutch *biennis,* has come down to us essentially unchanged from the times of Linnæus who gave us its name. We know of no plant better representative of a species of *Œnothera* and we know of no *Œnothera* which better satisfies the generally accepted requirement that a species should "breed true."

Œnothera biennis L. in large cultures comes so true that hundreds of plants may be grown without finding a single departure from the type. Yet Stomps ('14) in large cultures of selfed lines from a single wild plant collected in 1905 discovered that this Dutch *biennis* throws occasional marked variants ("mutants") and he described a *biennis semi-gigas* with the triploid number of chromosomes (21), a dwarf type *biennis nanella,* and a color variety *biennis sulfurea* with pale yellow petals. De Vries ('15) at once took up the study of certain of the lines established by Stomps and grew cultures which totaled 8,500 plants. Among these were 4 plants of *biennis semi-gigas* about 0.05 per cent., 8 plants of *biennis nanella* about 0.1 per cent., and 27 plants of *biennis sulfurea* about 0.3 per cent. Since the percentages from *Lamarckiana* are for *semi-gigas* 0.3 per cent. and for nanella 1 to 2 per cent. it should be noted that with respect to these "mutants" *biennis* appears to be the more stable of the two species, although the color variety *biennis sulfurea* constitutes a new type of variant in experimental studies on œnotheras. A culture of over 1,000 plants from selfed seed of *biennis sulfurea,* all with pale yellow flowers, produced 2 dwarfs thus establishing a "double mutant" *O. biennis* mut. *sulfurea* mut. *nanella.*

As evidence for the mutation theory of De Vries this behavior of the Dutch *biennis* is to the writer much more trustworthy evidence than the behavior of *Lamarckiana* for the reason that the latter plant in his opinion does not have a clear record of long existence, and probably is a form of comparatively recent origin. De Vries ('15, p. 173) has asserted again most vigorously his belief that *Lamarckiana* may be identified with a specimen from the United States collected by Michaux and now in the collections of the Museum d'Histoire Naturelle in Paris (De Vries, '14). With this view I cannot accord for reasons recently published (Davis, '15*a*). The showing of "mutants" from *Œnothera biennis* can hardly be considered very encouraging for the mutation theory of organic evolution when it is remembered that *biennis semi-gigas* is self sterile, that *biennis nanella* is frequently weakly or diseased, and that *biennis sulfurea* is clearly a retrogressive type having lost the power of producing normal yellow flowers.

Although *O. biennis* of all the œnotheras brought into the experimental garden still seems to me the form most free from suspicion of gametic impurity, nevertheless the line of Stomps has not, so far as we know, been subjected to the tests of a pure species summarized at the conclusion of this paper. De Vries ('15, p. 173) is mistaken in quoting me as conceding for this species a pure origin. I regard it simply as the safest material yet known on which to conduct studies in mutation, and with which other forms may be crossed to determine by the constitution of the F_1 hybrid generation whether or not their gametes are uniform. If in such a breeding test the F_1 progeny fall into two or more classes the assumption is justified that the form crossed with *biennis* must produce different classes of gametes. If the F_1 hybrid generation is uniform then it is clear that the functioning gametes male and female are respectively uniform. The fact that *Lamarckiana* crossed with *biennis* produces the "twin hybrids" *laeta* and *velutina* is, as has frequently been pointed out, one of the most important facts favoring the hybrid nature of *Lamarckiana*. It seems to me not improbable that other species of *Œnothera* will eventually be isolated more stable than the Dutch *biennis*.

Some exceedingly interesting observations have recently been reported by Bartlett ('15 *a, b, c*) on the behavior of certain small-flowered, self-pollinated American œnotheras. When grown in selfed lines these forms exhibit a behavior similar to that of *Lamarckiana* and *biennis* in throwing off in successive generations certain new types. Thus from one of the species, *Œnothera stenomeres,* a mutant *gigas* appeared with the diploid number of chromosomes, and from another species, *O. Reynoldsii,* certain individuals throw from 60 per cent. to 80 per cent. of dwarfs. It is too early to discuss the remarkable peculiarities of these forms since the material of Bartlett has not yet been tested for its purity along the lines presently to be discussed. Bartlett regards the new types as "mutants" in the sense of De Vries. The important point for our consideration at present is the fact that these wild plants apparently continue to reproduce themselves from generation to generation even while giving rise to the new forms.

With respect to the taxonomic status of the plants which we have just considered the writer sees no alternative but their recognition as clear species. The *Lamarckiana* of De Vries, the *biennis* of Linnæus, and most of the types which Bartlett has segregated from the American wild œnotheras breed true as to the mass of their progeny. What further qualifications can taxonomy in reason demand? Species they are by virtue of their morphology and by the test of the experimental garden which shows their characters to be stable to an extent that renders it certain that each line self-pollinated will maintain itself unchanged, indefinitely as far as we can see, through successive generations.

The argument that will follow as to the genetic constitution of these species of *Œnothera* does not in the least affect the matter of their recognition in taxonomy as species. It may be prefaced by two questions stated as follows: Are the types pure species, homozygous because the plants develop male gametes of one type only and because their female gametes have a uniform germinal constitution? Or, are the types heterozygous developing different types of male gametes and different types of female; briefly expressed have they in some degree a hybrid constitution?

But it will at once be asked, how can a species be hybrid even to a small degree and yet breed as true as do these forms under consideration? Where in their behavior is evidence of a hybrid constitution such as might appear in the splitting off of numerous different forms varying from the parent type, some in small degrees and some in larger degrees? Where is evidence of an orderly segregation of characters such as has been demonstrated by the Mendelian research of recent years? To these questions it must frankly be answered that only here and there are glimpses of situations which may possibly be interpreted in terms of Mendelian analysis. For example the characters of the "mutants" are frequently clearly retrogressive which indicates that gametes are formed lacking certain factors and suggests phenomena characteristic of segregation from heterozygous stock and very common in Mendelian behavior. Again, the repetition of the same "mutants" in a series of generations suggests a mechanism of precision such as we have come to associate with Mendelian inheritance. It is not, however, my purpose to argue at present this phase of the discussion for the experimental data before us is not in such shape that it can be handled to the best advantage. We admit that the "mutants" themselves do not establish their parents as in their nature hybrids. If they did there would of course be no discussion.

Under two conditions and apparently two only can a heterozygous species be conceived as breeding true.

First, if of the varied possible types of gametes *only such unite and produce fertile zygotes as will perpetuate the same germinal constitution as the parent,* then from such zygotes a heterozygous line might continue indefinitely as an impure or hybrid species. Under such conditions gametes which might in varied combinations give a series of different forms (segregates) are either not matured or if matured fail to function. Some degree of pollen and ovule sterility must be expected as the result of such conditions.

Second, if of a varied assortment of zygotes formed by the union of different types of gametes, *only those develop which have the germinal constitution of the parent* then again a heterozygous line might continue indefinitely and constitute a species, although impure or hybrid in its nature. Since all of the zygotes which result from other combinations of gametes either die or fail to develop beyond some early stage in the life history this condition would result in some degree of seed sterility or in the production of weak plants that must soon perish.

Now the œnotheras as a group exhibit a very remarkable amount of pollen sterility and also a high degree of ovule abortion, and these plants frequently give extraordinarily low yields of fertile seeds although seed-like structures may be formed in abundance. These facts we are just beginning to appreciate as offering problems for study. They seem to the writer of vital importance to the discussion of *Œnothera* genetics, facts which the Mutationists cannot ignore and behind which the Mendelians can maintain at present a very strong defence for their interpretations of the peculiarities of *Œnothera* behavior.

With respect to pollen sterility it has for many years been known that *Lamarckiana* and other species of *Œnothera* present large proportions of abortive pollen grains. Bateson (1902) early seized on the point and suggested that the high degree of pollen abortion in *Lamarckiana* indicated a hybrid plant exhibiting partial sterility. Geerts ('09) in an excellent account of the cytology of *Lamarckina* showed that approximately one half of the pollen grains fail to mature and that one half of the ovules fail to develop embryo sacs. Geerts ('09, p. 89) also made an examination of more than one hundred species of the Onagraceæ, giving us the conditions of pollen and ovulue fertility represented in some fifteen genera. He found generally in species of *Œnothera* and allied genera a degree of sterility similar to that in *Œnothera Lamarckiana,* about 50 per cent. for both pollen and ovules. On the other hand certain species of *Jussieua, Zauschneria, Epilobium, Boisduvalia* and *Lopezia* are wholly or almost wholly fertile.

My own examination of conditions in the material of *Œnothera* with which in recent years I have worked has shown some remarkable differences in the amount of pollen and seed sterility. Such close pollinated types as the Dutch *biennis,* the Dutch *muricata,* American *muricata* (from Woods Hole), *Tracyi,* and a number of American small-flowered species (for example *biennis* A and *biennis* D of my cultures (Davis, '11, p. 197 and '12, p. 385)), have very large amounts of sterile pollen. In the case of the Dutch *muricata* much more than 50 per cent. of the pollen has been sterile. Yet these are types which by virtue of their long history of close pollination might be expected to be among the purest of the species. On the other hand the race *grandiflora* B (Davis, '11, p. 203), and the western species *franciscana* and *venusta,* all open pollinated species show hardly more than a trace of pollen abortion, and *Jamesii* from Texas only a small amount of sterile pollen. I have this winter tested the seed fertility of some of these species by germinating the seeds in Petri dishes after the method recently described (Davis, '15*b*). The Dutch *biennis* gave a germination of about 96 per cent., the Dutch *muricata* about 72 per cent., *grandiflora* B about 95 per cent., *franciscana* about 61 per cent., *venusta* about 87 per cent., and *Jamesii* about 91 per cent.

It is interesting to note in the above list that the Dutch *biennis* with its very high percentage of fertile seeds (96 per cent.) has extensive pollen abortion and the Dutch *muricata* with seed germination of about 72 per cent. has an even lower degree of pollen sterility. On the other hand there are species of *Œnothera* with both high seed and pollen fertility as illustrated by some races of *grandiflora, venusta* and *Jamesii*. I was especially interested in the conditions shown by my race *grandiflora* B with its almost perfect fertility both as to pollen and seeds. This race isolated from a collection of mixed seeds gathered by Tracy in 1907 at Dixie Landing, Alabama, has always seemed to me to present a type of unusual purity. The line was started in 1908 by a cross of two similar plants (Davis, '11, p. 203) representing the broader-leaved forms of *grandiflora* that were present at Dixie Landing and I have grown in small cultures several generations of the plant without noting departures from the type. I cannot accept the criticism of De Vries ('14, p. 348) that my race *grandiflora* B is impure because from the same collection of mixed seeds of Tracy's he obtained a diversified culture as I also reported (Davis, '11, p. 203) when the line was first isolated, and because De Vries and Bartlett found the Dixie Landing station "desolate" five years after the visit of Tracy. This type may prove to be nearer to the desired pure species than the Dutch *biennis*.

Jeffrey in recent papers ('14*a*, '14*b*, '15) has taken the position "that in good species the spores or pollen is invariably perfect morphologically" and from this standpoint refuses to consider *Lamarckiana* and other œnotheras as suitable material on which to base experimental studies on mutations. To him the mere presence of abortive pollen suffices to stamp a form as hybrid in character. This represents an extreme view which in consideration of our ignorance of possible physiological reasons for pollen sterility can at present scarcely be claimed as more than an hypothesis. For the œnotheras we are greatly in need of cytological and physiological studies on pollen sterility more detailed than the incidental observations that have so far been published.

With respect to the abortion of ovules among the œnotheras our information is practically confined to the observations of Geerts ('09), mentioned above. It appears that in *O. Lamarckiana* and a number of other species only about 50 per cent. of the ovules develop embryo sacs. Other species also show varying degrees of ovule abortion. The ovules that fail to mature are represented in the capsules by a fine light brown powder known to all who work with œnotheras. Such powder is very common in the capsules of various species and their hybrids, and it seems probable that ovule sterility is as widespread in this group of plants as is the degeneration of the pollen. As in the case of pollen sterility we do not know to what extent physiological conditions may also be responsible for the abortion of ovules.

Pollen and ovule sterility involve of course the elimination from the life history of immense numbers of gametes and raise the following questions. Can it be that this elimination throws out of the life cycle types of gametes with germinal constitutions different from the gametes that matured and that function? It is possible that some of the *Œnotheras* species, in hybrid condition, regularly mature for the most part particular classes of gametes which in conjugation will perpetuate the genetic line of the parent plant? Gametes even when normally developed may still not function as when pollen grains fail to germinate upon the stigma because its secretions are not suitable. It must also be borne in mind that there are yet other phases of the life history when gametes may become ineffective as through failure to conjugate or because of a high mortality among zygotes, embryos, or young plants; such forms of infertility are expressed in sterile seeds or in weak offspring which never mature. Possibly the so-called "mutants" arise when unusual gametes from hybrids, occasionally surviving the extensive process of degeneration, form zygotes also able to survive and to develop plants diverging from the parents.

The subject of seed sterility among the œnotheras has scarcely been touched by the students of the group and yet it seems likely to become a factor of prime importance in its bearings on the problems of *Œnothera* genetics. Any worker among these plants shortly becomes aware of the fact that very many of the seed-like structures which he sows fail to germinate even though seed pans are kept for many weeks. De Vries makes frequent reference to the facts of seed sterility and the writer has in recent years recorded the number of seeds sown in cultures and the number of seedlings that develop. The results are most surprising and must have significance although what that may be remains for the future to disclose. A line of research has opened before us that will demand a special technique, for it is not enough to know merely that certain proportions of the seeds germinate within the time practicable for keeping seed pans under observation.

Seed-like structures sown on the earth are obviously lost for further enquiry as to the facts of their viability; a proportion of seedlings appear but as for the residue, that cannot be examined. The residue may contain viable seeds the germination of which is delayed, or it may consist wholly of sterile structures. We must develop methods that will ensure the rapid and complete germination of seeds in convenient receptacles such that the residue of sterile structures may be left for study after the seedlings have been removed and set in the earth. By such methods cultures of *Œnothera* may be grown in which one may feel confident that all of the viable seeds have germinated since by an examination of the residue it may be determined whether or not the seed-like structures have embryos. It is probably safe to say that no culture of *Œnothera* has as yet been described in which we may feel certain that the progeny of the sowing is complete. During the past winter I have tested the percentage of seed fertility in some fifty species and hybrids of *Œnothera* germinating the seeds on pads of wet filter paper in Petri dishes. With this method may advantageously be combined the clever practical suggestion of De Vries ('15, p. 190) of forcing water into wet seeds by air pressure thereby greatly hastening their germination. A description of a method of seed germination which will, I think, prove to be satisfactory in genetical work on *Œnothera* may be found in the Proceedings of the National Academy of Sciences, Vol. I., p. 360, 1915.

The first investigator to make use of the facts of seed sterility in suggesting Mendelian interpretations of the behavior of *Lamarckiana* and certain *Œnothera* crosses has been Renner ('14) and his line of investigation has opened a field of research and speculation that must be reckoned with in the future. Renner has studied the seed structure in *Lamarckiana, biennis* and *muricata,* and in certain crosses among these forms. His conclusion on the genotype of *Lamarckiana* will illustrate the principles underlying the method of attack. Since *Lamarckiana* when crossed with *biennis* and certain other species gives in the F_1 hybrid generation the twin hybrids *læta* and *velutina* it may be assumed to develop two classes of gametes which function. These may be spoken of as the *læta* and *velutina* gametes and are produced in about equal numbers. When *Lamarckiana* is self-pollinated the *læta* and *velutina* gametes may combine in proportions to give 1 pure *læta*: 2 *læta-velutina*: 1 pure *velutina*. It is a fact that more than one half of the seeds of *Lamarckiana* fail to develop normal embryos and Renner concludes that these sterile seeds represent zygotes homozygous respectively for the *læta* and *velutina* factors. The fertile seeds develop from the heterozygotes with both *læta* and *velutina* factors combined and this combination gives the characters of *Lamarckiana. Œnothera Lamarckiana* may thus be an impure or heterozygous species breeding true because of the death of such zygotes as carry the factors for *læta* and *velutina* in homozygous conditions. This simple Mendelian explanation of the behavior of *Lamarckiana* points a line of interpretation and study certain to be fruitful in *Œnothera* research.

Among hybrids of *Œnothera* the seed sterility sometimes runs extraordinarily high. The most remarkable illustrations of this fact so far known appear in the second generations of crosses involving the Dutch *biennis* and the Dutch *muricata* which exhibit certain remarkable morphological peculiarities discovered and described by De Vries ('13). First generation hybrids of reciprocal crosses between these species grown by the writer in 1913 gave data on seed germination in the earth as presented in Table I.

TABLE I.

F_1 Hybrids of Reciprocal Crosses between *O. biennis* and *O. muricata.*

Culture.	Cross.	Seeds Sown.	Sown in	Seedlings.	Germination.	Duration of Experiment.
13.33	F^1 *biennis* × *muricata*	673	Earth	139	20%	6 weeks
13.34	F^1 *muricata* × *biennis*	153	Earth	97	63%	7 weeks

It is probable from my experience with other species crosses that the viability of the seeds of these F_1 hybrids is really high and that the relatively low percentages recorded above are due to de-

TABLE II.

F_2 Hybrids of Reciprocal Crosses between *O. biennis* and *O. muricata,* Including Certain Double Reciprocals, Sesquireciprocals, and Iterative Hybrids.

Culture.	Cross.	Seeds Sown.	Sown in	Seedlings.	Germination.	Duration of Experiment.
14.41 (13.33a)	F_2, *biennis* × *muricata*	466	Earth	8	1.7%	9 weeks.
14.42 (13.34c)	F_2, *muricata* × *biennis*	205	Earth	35	12%	9 weeks.
14.43 (13.33a × 13.34)	double reciprocal (*b* × *m*) × (*m* × *b*)	73	Earth	8	11%	9 weeks.
15.31 (14.33 × 14.16)	sesquireciprocal (*b* × *m*) × *b*	267	Earth	25	9%	9 weeks.
*15.31 (14.33 × 14.16)	sesquireciprocal (*b* × *m*) × *b*	282	Petri dish	132	46%	6 weeks.
15.32 (14.16 × 14.33)	iterative *b* × (*b* × *m*)	22	Earth	1	4%	9 weeks.
15.33 (14.33 × 14.20)	iterative (*b* × *m*) × *m*	212	Earth	2	0.9%	9 weeks.
*15.33 (14.33 × 14.20)	iterative (*b* × *m*) × *m*	292	Petri dish	42	14%	7 weeks.
15.34 (14.34 × 14.16)	iterative (*m* × *b*) × *b*	217	Earth	47	21%	9 weeks.
*15.34 (14.34 × 14.16)	iterative (*m* × *b*) × *b*	373	Petri dish	73	19%	4 weeks.
15.35 (14.34 × 14.20)	sesquireciprocal (*m* × *b*) × *m*	246	Earth	43	17%	9 weeks.
*15.35 (14.34 × 14.20)	sesquireciprocal (*m* × *b*) × *m*	498	Petri dish	198	39%	7 weeks.
15.36 (14.20 × 14.34)	iterative *m* × (*m* × *b*)	198	Earth	51	25%	9 weeks.

layed germinations. But the figures for germination in the earth of F_2 hybrids and of double reciprocals, sesquireciprocals, and iterative hybrids are most surprising in the degree of sterility or delayed germination shown. They are given in Table II., where are also presented the records of four cultures sown in Petri dishes in which the germination was complete as proved by an examination of the residue.

A comparison in Table II. of the record for culture 15.31 with *15.31, 15.33 with *15.33, and 15.35 with *15.35 will illustrate the gain in germination that may come through sowing seeds in Petri dishes. The percentages of germination presented above for the hybrids of *biennis* and *muricata* must not be regarded as expressing exactly the degree of seed fertility under the conditions of the experiments since with the harvests of seed are frequently found very many structures too large to be abortive ovules and too small to be counted as "seeds" in the sense of falling within the limits of seed size. These structures are probably undeveloped seeds but only a microscopical examination can determine this point; if so, their presence of course always lowers the percentage of zygotes capable of giving progeny.

Bearing in mind the fact that pollen sterility in *biennis* and *muricata* is 50 per cent. or more and that pollen abortion in the F_1 hybrids is very much higher (in fact very little good pollen is produced) the total amount of sterility both gametic and zygotic is simply amazing. Under such conditions how can the behavior of these hybrids be looked upon as indicative of anything but a most unusual situation, in itself very interesting, but far beyond the expectations of normal hybrid behavior. This remarkable degree of sterility among the hybrids of *biennis* and *muricata* is perhaps extreme for the œnotheras, but it serves to illustrate conditions extensively present in the writer's experience and doubtless also in the experience of others.

De Vries has described the hybrids between *biennis* and *muricata* as breeding approximately true which in the main has also been my observation. Apparently largely upon this behavior and that of certain other crosses he has reached the conclusion that hybrids between species of *Œnothera* are stable. In this opinion of De Vries I cannot agree for my crosses between *grandiflora* and certain small-flowered American species (Davis, '12 and '13), and between *biennis* and *franciscana* have in the F_2 generations given abundant evidence of that extensive variation interpreted as segregation. I believe that the apparent stability of the very small progenies produced by hybrids of *biennis* and *muricata* simply means that the remarkably high mortality among gametes and zygotes of these hybrids, or the delayed germination of their seeds, has prevented the appearance in our cultures of the diverse types which theoretically would be expected. Any general conclusions on genetic behavior in the œnotheras which fails to take into account the phenomena of sterility rests upon insecure foundations.

It is true that we do not know to what extent physiological factors may affect seed sterility as well as pollen and ovule abortion. Nevertheless a main fact is clear, namely that seed sterility eliminates in certain *Œnothera* species and hybrids immense numbers of zygotes which fail to develop seeds. And, furthermore, we know for œnotheras that large classes of weak offspring are sometimes produced that are unable to reach maturity. Seedlings with white or yellow cotyledons, which quickly die, are not uncommon in my experience with *Œnothera* cultures; in certain cases they have appeared in very large numbers (Davis, '11, p. 222) and probably have important genetical significance. This situation in *Œnothera* finds a close parallel in the behavior recorded for a number of animals and plants. Thus Baur's "golden" variety of *Antirrhinum* is an impure or heterozygous form which besides reproducing itself throws a class of normal green plants and a class represented by weak yellow seedlings that shortly die. The yellow mice studied by Castle and Little although interbred always remain impure giving progeny heterozygous for yellow because of the death of zygotes with a double dose of the factor for yellow. A dwarf wheat isolated by Vilmorin cannot be fixed since it always remains heterozygous throwing talls but never producing homozygous dwarfs. The white female form of the clover butterfly, *Colias,* was found by Gerould always to give yellow offspring either because of the failure of the gametes carrying white to conjugate or because zygotes homozygous for white fail to develop. A form of *Drosophila* characterized by confluent wings has been found by Metz only in the heterozygous condition, always throwing normals and never breeding true; flies homozygous for confluent wings are apparently not viable. Is it not possible that parallel or related phenomena are extensively present among the œnotheras? The mortality as shown by sterile seeds may indicate the elimination of large groups of forms divergent from the parent types, and some of the curious dwarfs and aberrant plants which again and again have been reported in *Œnothera* lines may be from zygotes barely able to survive the death-producing conditions that eliminate so many of their companions.

So far we have considered evidence chiefly of a negative character for the contention that many of the species of *Œnothera* are impure or hybrid species. We have tried to show that pollen, ovule, and seed sterility must all be reckoned with as conditions which may eliminate Mendelian classes of gametes and hold a line to a history of relatively true breeding even though the stream of germ plasm remain heterozygous or impure in character. The natural corollary of such behavior, if proven, might be the interpretation of so-called "mutants" as segregates from a hybrid stock that were able to survive the destruction meted out by conditions that produce sterility. To what extent the causes of sterility may lie in the history of gametogenesis or may be due to unfortunate combinations of gametes, or to what extent sterility is the result of physiological factors, these are problems that lie before us.

Let us now examine some positive evidence that certain species of *Œnothera* do form distinct classes of gametes and in consequence seem likely to be heterozygous in their constitution. That which first demands attention is the situation discovered by De Vries in certain first generation hybrids and by him named "twin hybrids." We have already referred to this phenomenon first described by De Vries ('07) for the behavior of *Lamarckiana* which as a pollen parent in crosses with other species of *Œnothera* gives not uniform F_1 generations but the two types *læta* and *velutina* (twin hybrids), produced in about equal numbers. Certain "mutants" of *Lamarckiana* also give twin hybrids under the same conditions as those produced by *Lamarckiana*. The behavior is so exact that the simplest hypothesis must suppose that *Lamarckiana* and these "mutants" form two classes of gametes which are fertile in these particular crosses. De Vries ('09) has also described "triple hybrids" when the "mutants" *scintillans* and *lata* are pollinated by such species as produce the twin hybrids from *Lamarckiana*. In such cases two of the forms have the characters of *læta* and *velutina* combined with those of the other parent, and the third form resembles the mother, either *scintillans* or *lata*. The phenomena of twin and triple hybrids is treated in detail by De Vries ('13) in "Gruppenweise Artbildung."

From a Mendelian standpoint the production of twin and triple hybrids is strong evidence that *Lamarckiana* and such of its "mutants" as behave in this manner are impure or hybrid since the male or female gametes are not uniform, a point which has been emphasized by several critics of the mutation theory. De Vries assumes that *Lamarckiana* forms its different classes of gametes as a result of its mutating instability but the precision of the process falls completley in line with what we know of Mendelian behavior. The remarkable studies of Shull show that crosses between *Lamarckiana* and *cruciata* give in the first generation polymorphic progenies of much greater complexity than the twin hybrids of De Vries. Shull's results have not been published in full but, as I understand them, they indicate the interaction of several classes of gametes, a condition very far from what would be expected if genetically pure species had been crossed.

Very interesting are the observations of Atkinson ('14) on first generation crosses between *Œnothera nutans* and *O. pycnocarpa*. These two forms are American species recently segregated by Atkinson and Bartlett from the *biennis* alliance. They have bred true in garden cultures. When *pycnocarpa* is pollinated by *nutans* twin hybrids appear in the first generation. In the reciprocal cross *nutans* × *pycnocarpa* the same twin forms are produced and in addition a third type, making this generation a compound of three distinct forms, triple hybrids. Atkinson, apparently confident of the genetic purity of *nutans* and *pycnocarpa* assumes that the determination of the twin and triple hybrids takes place through a differential division in the zygote by which factors representing certain characters are side tracked in the suspensor cell and only those responsible for the twins and triplets pass on to the embryo. There is no cytological evidence that the first mitosis in the zygote of a higher plant is ever a differential division. To the writer the situation indicates that one or both of the two species is heterozygous and that for this reason classes of gametes are formed, appropriate combinations of which give the twins and triplets. No data has been published respecting the sterility of these two species, either of pollen or ovules, and nothing of seed abortion. An understanding of the genetic constitution of the species is likely to be a difficult matter, but it does not seem probable that both are pure.

What shall be said of the probable purity of the plants of *Œnothera* and *Raimannia* with which MacDougal worked in his experiments designed to create new species by the injection of certain fluids into the ovaries. The parent material was reported to breed true, but the cultures were small and not long continued and there is no reason to suppose that a complete germination of the seeds was obtained. No information is given on the fertility of the species either with respect to the abortion of gametes or the proportion of good seeds. The material was not tested by cross breeding with other forms (the purest known) to determine whether the F_1 hybrids were uniform, a most necessary test in the establishment of a stock as homozygous. Thus from our present viewpoint we cannot accept MacDougal's conclusion since the probabilities are very great that the new types which appeared in his cultures were produced not as the result of the injections but because of the genetic impurity of the plants themselves.

In the above discussion the writer has taken definitely a Mendelian attitude in sympathy with the criticisms of Bateson and the studies of Heribert-Nilsson ('12) and of Renner ('14). There are constant suggestions of order in the phenomena of inheritance among the œnotheras which while they may not fall into simple schemes of Mendelian notation nevertheless do indicate system even though masked by complexities. That the complications at least in great part are due to the genetic impurity of the *Œnothera* material which has been so far the subject of study is the writer's belief. The difficulties that surround the analysis of *Œnothera* inheritance are probably in very large measure due to the extraordinary amount of sterility, gametic or zygotic, or both, that is present in the group.

Upon students of this genus rests the responsibility of obtaining data on this sterility and, if possible, of discovering its causes. The assumption that a line represents a pure species because it breeds true is not a safe foundation upon which to conduct experimentation in the œnotheras. This is the assumption upon which have been based many of the conclusions of the Mutationists, and from it we must dissent. We cannot depart from the principles underlying Mendelian methods of research which have so brilliantly opened the present century of biological investigation.

Finally what are the tests that must be applied to an *Œnothera* species to determine whether or not it is pure.

First.—There is the breeding test and that must be applied with such experimental methods of seed germination (Davis, '15) as will insure a complete progeny from the sowing, a progeny wholly representative of all types of viable seeds. Even then the breeding test is negative rather than affirmative in its conclusions. Should the form throw off numerous variants it naturally becomes a subject of suspicion, but should it breed true or relatively true that does not in this group of plants prove it to be homozygous in its germinal constitution.

Second.—Information must be obtained on the character and degree of sterility present, both gametic and zygotic. Sterility, unless shown to be strictly physiological in its character, suggests genetic impurity.

Third.—Cross-breeding tests must be planned and followed in which the form under observation is mated with material of known genetic purity. If the hybrid plants of the first generation are essentially uniform and the result of a normal germination of the seeds the indications are strong that the form is truly pure provided that the gametes are likewise normally fertile. If the hybrids of the first generation fall sharply into classes the material must develop gametes of different germinal constitutions and is consequently heterozygous. One favorable cross with a pure species may not be sufficient to establish the purity of a form; a number of favorable tests with pure types will carry increasing conviction.

It is thus not an easy matter to determine the fact whether or not a species of *Œnothera* is pure, and yet this is fundamental to experimental studies in the group. On the assumption of specific purity the Mutationists rest their conclusions. This condition with respect to the characters studied is also basic to Mendelian experimentation. It need scarcely be emphasized that no species of *Œnothera* has as yet passed the tests for genetic purity outlined above and that consequently we have at present no standard material with which forms may confidently be mated in the test of cross-breeding. It should become the concern of *Œnothera* geneticists to find and isolate pure material as the starting point of further studies in experimental morphology. Whether such pure forms will be found among the wild species or as products of the garden time will determine.

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